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The Patent Office

Concept House
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1. Your reference — ANTT / P25314GB

2. Patent application number *(The Patent Office will fill in this part)* — 12 JAN 2002 — 0200657.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Antisoma plc
West Africa House, Hanger Lane
Ealing
London W5 3QR
United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom — 8302515001

4. Title of the invention

CANCER TREATMENT

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ERIC POTTER CLARKSON
PARK VIEW HOUSE
58 THE ROPEWALK
NOTTINGHAM
NG1 5DD

Patents ADP number *(if you know it)* — **1305010**

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) *any applicant named in part 3 is not an inventor; or*
b) *there is an inventor who is not named as an applicant, or*
c) *any named applicant is a corporate body. See note (d))*

YES

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9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	9
Claims(s)	2
Abstract	1
Drawing(s)	2

10. If you are also filing in any of the following, state how many against each item.

Priority Documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	NO
Request for preliminary examination and search *(Patents Form 9/77)*	YES
Request for substantive examination *(Patents Form 10/77)*	YES
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: E C Potter Clarkson

Date: 11 January 2002

ERIC POTTER CLARKSON

12. Name and daytime telephone number of person to contact in the United Kingdom: **0115 9552211**

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CANCER TREATMENT

The present invention relates to materials and methods for the treatment of cancer. In particular, the invention relates to a therapy comprising the administration of a radiolabelled antibody, which binds selectively to polymorphic endothelial mucin (PEM) in combination with a chemotherapeutic agent.

DeNardo *et al.* (April 1997) *P.N.A.S. USA* **94** pp. 4000-4004 mention that a synergistic therapeutic effect can be obtained in a mouse model of breast cancer by administering a yttrium 90-labelled chimeric L6 antibody (^{90}Y-ChL6) 6 or 24 hours before Taxol® (paclitaxel) administration. However, no synergistic effect was observed when Taxol® was administered 24-27 hours before ^{90}Y-ChL6.

DeNardo *et al.* conclude as follows: ^{90}Y-ChL6 and Taxol® can be given in a sequence that enhances therapeutic efficacy. Over time, after injection, ^{90}Y-ChL6 binds to malignant cells as it circulates and unbound ^{90}Y-ChL6 is cleared from normal tissues. Thus a "window" in time exists when there is ongoing tumour irradiation but little concurrent normal tissue irradiation. Given in this window, Taxol®, a small molecule rapidly taken up by the tumour, enhances the therapeutic effect of ^{90}Y-ChL6 on targeted malignant cells. The optimum time for Taxol® administration is 6-24 hours after ^{90}Y-ChL6.

ChL6 consists of a human IgG constant region and the Fab' region of murine monoclonal antibody (mAb) L6. ChL6 reacts with an integral membrane glycoprotein expressed at a high frequency on human breast, colon, ovary and lung carcinomas.

Gillies, at "Magic bullets: an update on therapeutic antibodies", London 27th to 28th June 2001, reported that CT26-EpCAM subcutaneous tumours treated with ^{125}I-KS-IL2 (iodine-125 labelled KS antibody IL-2 fusion) and Taxol® showed the two treatments to have a synergistic effect when the immunotherapy was given 24 hours after the chemotherapy. From this he concluded that "Optimal chemotherapeutic doses may lower tumour interstitial pressure and increase targeting of immunocytokines", in other words the chemotherapy e.g. Taxol®, should be administered before the immunotherapeutic.

Thus, both DeNardo and Gillies suggest that the order of administration is important to the efficacy of a combined chemotherapy and immunoradiotherapy. However they disagree on the most useful order of administration for the chemotherapeutic and immunotherapeutic agents.

The search for anti-cancer agents and methods of treatment is ongoing and intense. The present invention seeks to provide further agents and methods for the treatment of cancers.

Summary of the invention

The inventor has discovered that a synergistic tumouricidal effect can be obtained by means of a combined treatment with a radiolabelled antibody that binds selectively to polymorphic endothelial mucin (PEM), and a chemotherapeutic agent.

PEM is a component of the human milk fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

The preferred chemotherapeutic agent is the antineoplastic agent Taxotere® (Docetaxel), which is a semi-synthetic analogue of Taxol®. For an overview of Taxotere® see J L Fabre *et al.* (1995) *Drugs Future*, **20**, pp. 464-471. For synthesis and structure see M Colin *et al.* US 4924012 (to Rhône-Poulenc Sante). For anti cancer activity see Riou *et al.* (1992) *Biochem. Biophys Res. Commun.* **187**, pp. 164-170. Taxotere® is available commercially from Rhône-Poulenc Rorer.

Monoclonal antibodies that will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982) and *"Antibody Engineering, A Practical Approach"*, J McCafferty *et al.*, ed. (IRL Press, 1996).

WO 01/74905 discloses antibodies that bind selectively to PEM and are useful in accordance with the present invention.

Preferably, the antibody is HMFG-1, which is available from Imperial Cancer Research Fund, England. More preferably the antibody is a humanised HMFG-1. Such antibodies are disclosed in WO 92/04380.

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimiriou *et al.*, (1981), *Int. J. Cancer* **28** pp. 17-21 and Gendler *et al.*, (1988), *J. Biol. Chem.* **236** pp. 12820-12823).

HMFG-1 monoclonal antibodies bind to a particular component of HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the

amino acid sequence APDTR within the twenty amino acid tandem repeats of the *muc-1* gene product.

By 'humanised antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complementarity-determining region (CDR) is derived from a second, donor monoclonal antibody that may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably both chains of the humanised monoclonal antibody CDRs are grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (*i.e.* humanised) chain comprises two or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human framework residues and CDRs from a donor antibody having specificity for PEM.

However, it will be appreciated by those skilled in the art that in order to maintain and optimise the specificity of the humanised antibody it may be necessary to alter one or more residues in the framework regions such that they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are derived from an human IgG monoclonal antibody.

Methods of making humanised monoclonal antibodies are well-known in the art, for example see Jones *et al.* (1986) *Nature* **321** pp. 522-525, Riechmann *et*

al. (1988) *Nature* **322** pp. 323-327, Verhoeyen *et al.* (1988) *Science* **239** pp. 1534-1536 and EP 239 400 (to Winter).

By "in combination with one another" regarding the antibody and chemotherapeutic agent treatments we include the meaning not only that the antibody and chemotherapeutic agents are administered simultaneously, but also that they are administered separately and sequentially.

By "binds selectively" we include the meaning that the antibodies in question will specifically bind cells displaying PEM on their surface and will not bind to those cells not displaying PEM.

An example embodying an aspect of the invention will now be described with reference to the following figures in which:

Figure 1 shows the effect of various treatments on tumour volume in a human derived bladder cancer cell line subcutaneously implanted on a mouse.

Figure 2 shows the effect on tumour tripling times of various treatments.

Example: Combination therapy increases tumour-tripling times significantly.

Materials & methods

Cell lines

The human bladder tumour cell line, HT1376, expressing polymorphic epithelial mucin (PEM) was cultured in RPMI 1640 tissue culture medium containing 100 $U \cdot ml^{-1}$ penicillin and 100 $\mu g \cdot ml^{-1}$ streptomycin, supplemented with 10% foetal calf serum in a humidified atmosphere of 5% carbon dioxide in

air. HT1376 is a human bladder carcinoma cell line obtained from the European Collection of Animal Cell Cultures (ECACC no. 87032402).

Antibody

The fully humanised version of the anti-PEM antibody, HMFG1, was produced by Lonza, Slough, UK. This humanised HMFG1 (hHMFG1) was conjugated with the chelating agent CITC-DPTA by BioInvent, Sweden.

Radiolabelling

CITC-DTPA-conjugated hHMFG1 was radiolabelled with ^{90}Y in acetate buffer (pH 5.5) at room temperature for 30 minutes. Disodium EDTA was added to the reaction mixture such that the final EDTA concentration was 5 mM and left to stand at room temperature for approximately 10 min. The radiolabelled protein was then purified by size exclusion chromatography and the protein-containing fractions pooled.

Animal model

Mice

Female MF1 athymic nude (nu/nu) mice were used throughout these studies. The mice were bred at the Biological Research Facility of St. George's Hospital Medical School and were housed in sterile filter cages and maintained on irradiated diet and sterile water. Tumours were established by subcutaneous injection of 5×10^6 cells in the right flank.

Tumour therapy

Approximately three weeks after tumour inoculation, when the tumours were around 0.2 cm^3 in volume (7-8 mm in diameter), mice were divided into treatment groups of 7-8 mice each. The tumour volumes in each group at the time of treatment were not significantly different. Mice were injected with various doses of ^{90}Y-labelled hHMFG1 radioimmunotherapy (10-20 μg, 1.2-

2.0 MBq) either alone or in combination with 10 mg/kg Taxotere® given either 24 h before or 24 h after radioimmunotherapy, or with chemotherapy or chemotherapy vehicle alone. One group of mice was left untreated as a control on some occasions when experimental animals were treated. Tumour diameters (d_1, d_2 and d_3) were measured twice weekly in three orthogonal directions using a vernier calliper and the tumour volume (v) calculated according to the formula for an ellipsoid:

$$v = \frac{\pi}{6}(d_1 \cdot d_2 \cdot d_3)$$

Tumour measurement commenced one week before treatment and continued until the tumours had at least tripled in volume. Relative tumour volume (the volume of each tumour divided by the tumour volume on the day of treatment) was calculated to minimise the effect of variation in treatment volume of the individual tumours. The end-point was defined as time for the relative tumour volume to reach 3.

Statistics

The Wilcoxon rank sum test was used to compare the groups of mice receiving the various treatment protocols. A p-value <0.05 was considered to be significant.

Results

HT1376 volume tripling times (days)

^{90}Y-HMFG1 plus chemotherapy

Treatment	Median	T-C
Control (18-19)	17.9	
Taxotere® 10mg/kg (15)	43.8	25.9
1.2 MBq ^{90}Y-hHMFG1	23.4	5.5
2.0 MBq ^{90}Y-hHMFG1	46.5	28.6
1.2 MBq + Taxotere®	73.3	55.4
Control (25-26)	19.0	
Taxotere® 10 mg/kg (27-31)	41.2	22.2
Dox 10 mg/kg (20-22)	52.9	33.9
Taxol® 10 mg/kg (23)	30.2	11.2
Control (76-79)	21.7	
1.6 MBq ^{90}Y-hHMFG1	40.6	18.9
Tax + dox 5 mg/kg each	35.9	14.2
1.6 MBq + chemo	49.8	28.1
Chemo + 1.6 MBq (80)	50.8	29.1
Control (93-96)		
1.6 MBq ^{90}Y-hHMFG1		
Taxotere® 10 mg/kg		
1.6 MBq + Taxotere®		
Taxotere® + 1.6 MBq		

Table 1 summarises the treatments given and the results obtained. The value T-C is the tumour tripling time of the test treatment minus that of the control in which no treatment was given. Hence, the larger the T-C value, the better the therapeutic effect obtained. Figures 1 & 2 represent graphically the results obtained.

From Table 1 it is clear that a combination of 1.2 MBq ^{90}Y-hHMFG1 and Taxotere® increased the tumour tripling time more than twice as much as Taxotere® on its own and ten times as much as the same dose of ^{90}Y-hHMFG1 on its own. In fact, the contribution of radiolabelled antibody and Taxotere® was almost twice as effective (T-C = 55.4) as the additive effect of the individual treatments (T-C = 5.5 + 25.9 = 31.4).

Table 1 also shows that the sequence of treatment with ^{90}Y-hHMFG1 and Taxotere® does not alter the effectiveness of the treatment significantly. Administration of 1.6MBq ^{90}Y-hHMFG1 before Taxotere® gave a T-C value of 28.1 whereas administration in the reverse order gave a T-C value of 29.1.

CLAIMS

1. A therapeutic system for the treatment of tumours comprising a combination of component (i) a radiolabelled antibody and component (ii) a chemotherapeutic agent, wherein the antibody binds selectively to polymorphic endothelial mucin (PEM); the components (i) and (ii) being provided for use in the treatment of a tumour wherein the radiolabelled antibody and a chemotherapeutic agent are administered in combination with one another.

2. A therapeutic system for use as claimed in Claim 1 wherein the chemotherapeutic agent is Taxotere® (docetaxel).

3. A therapeutic system for use as claimed in Claim 1 or 2 wherein the antibody treatment precedes treatment with the chemotherapeutic agent.

4. A therapeutic system for use as claimed in Claim 1 or 2 wherein the chemotherapeutic agent treatment precedes treatment with the antibody.

5. A therapeutic system as claimed in any of Claims 1 to 4 wherein the antibody is humanised.

6. A therapeutic system as claimed in Claim 5 wherein the antibody is HMFG-1.

7. A therapeutic system as claimed in any preceding claim wherein the chemotherapeutic agent is selected from at least one of Taxotere®, Taxol®, Doxorubicin and Cisplatin.

8. A therapeutic system as claimed in any of claims 1 to 10 wherein the tumour is associated with at least one of the following disorders: breast cancer, ovarian cancer, lung cancer, gastric cancer and bladder cancer.

ABSTRACT

Cancer Treatment

The invention relates to a therapeutic system comprising (i) a radiolabelled antibody, which binds selectively to polymorphic endothelial mucin (PEM) such as the monoclonal antibody HMFG-1, and (ii) a chemotherapeutic agent, such as Taxotere®. The radiolabelled antibody and chemotherapeutic agent are administered in combination with one another to produce a synergistic therapeutic effect.

Fig 1

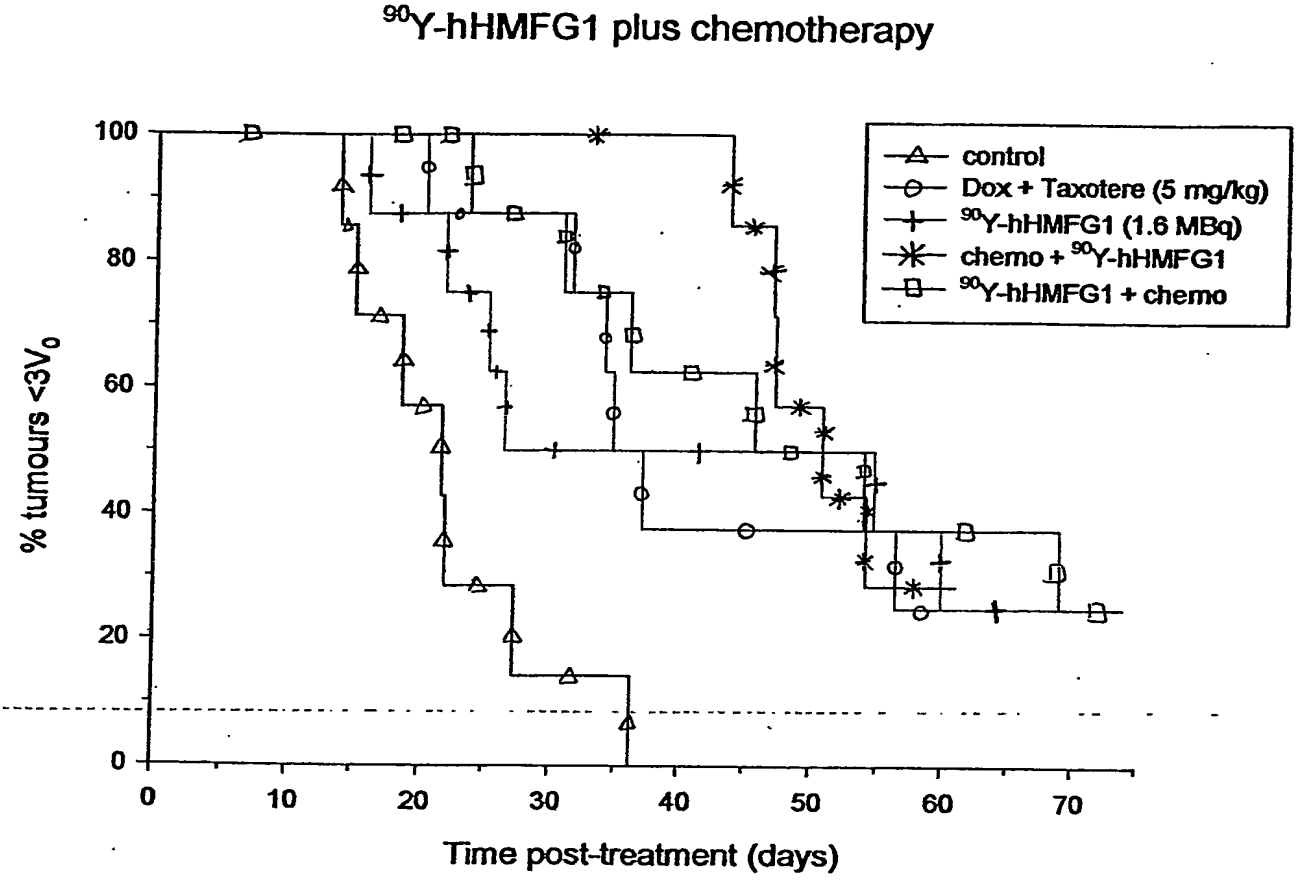
^{90}Y-hHMFG1 plus chemotherapy
% tumours <3V$_0$
Time post-treatment (days)
control
Dox + Taxotere (5 mg/kg)
^{90}Y-hHMFG1 (1.6 MBq)
chemo + ^{90}Y-hHMFG1
^{90}Y-hHMFG1 + chemo
0
20
40
60
80
100
0
10
20
30
40
50
60
70

Figure 1

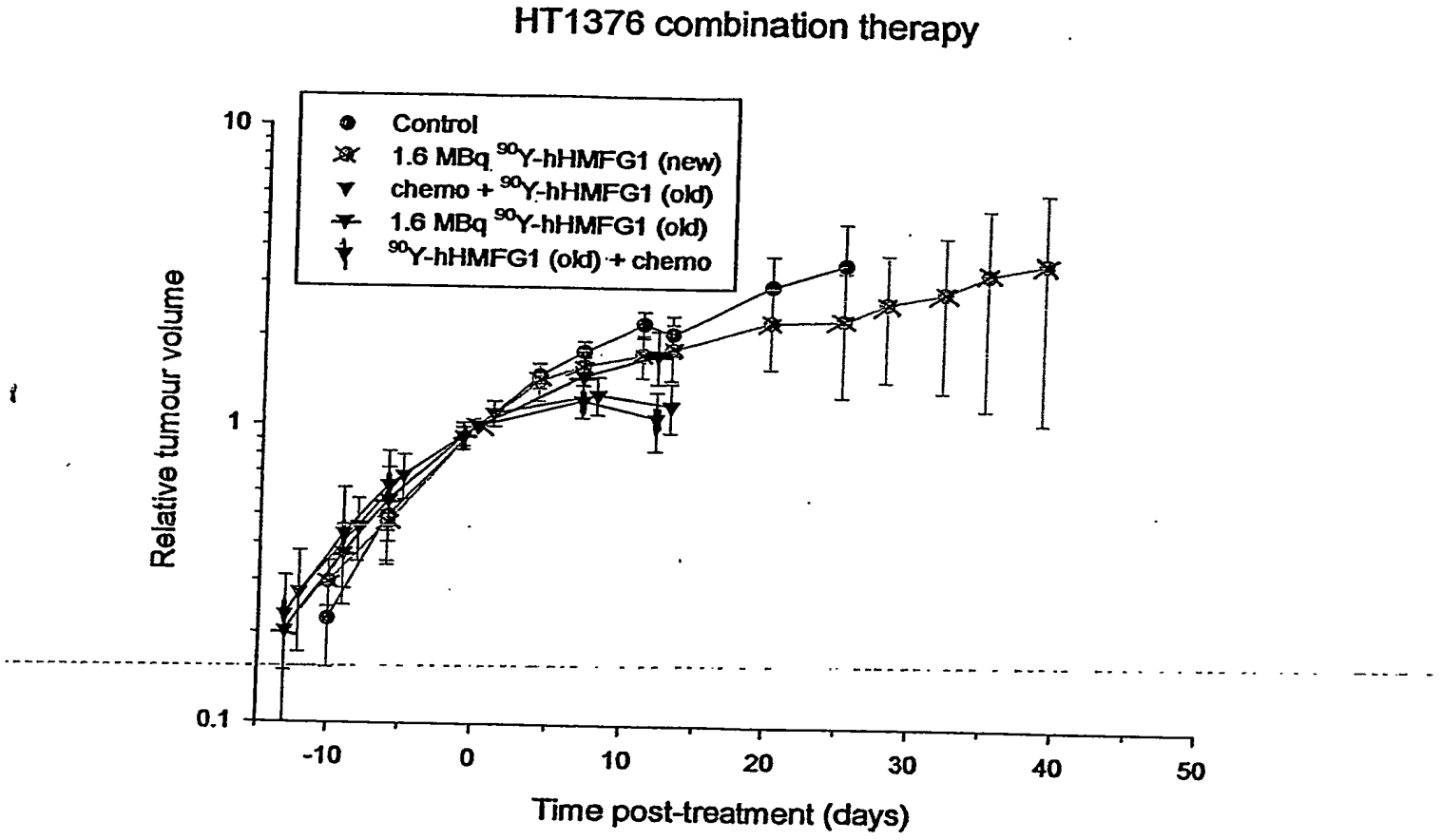
HT1376 combination therapy
Control
1.6 MBq ^{90}Y-hHMFG1 (new)
chemo + ^{90}Y-hHMFG1 (old)
1.6 MBq ^{90}Y-hHMFG1 (old)
^{90}Y-hHMFG1 (old) + chemo
Relative tumour volume
10
1
0.1
-10
0
10
20
30
40
50
Time post-treatment (days)

Figure 2